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DNA extraction from apple leaves or apple buds V.1

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We use this protocol and it's working

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Abstract

This protocol describes a DNA purification step prior to following the standard workflow of the Macherey-Nagel NucleoSpin Plant II kit for plant genomic DNA extraction.

It is specifically designed for frozen and mechanically disrupted plant tissue, allowing efficient removal of contaminants such as polysaccharides, secondary metabolites, and cell wall debris that may interfere with downstream silica column binding.

The procedure begins with cryogenic grinding using a Qiagen TissueLyser II (or III), followed by chemical lysis using Carlson buffer and RNase A. A chloroform extraction is then performed to separate the aqueous phase from organic impurities. The supernatant is combined with PC buffer and applied to silica spin columns, followed by a series of ethanol-based wash steps and elution of purified DNA.

This enhanced pre-treatment improves the yield and purity of DNA from difficult or complex plant tissues and is fully compatible with the manufacturer's protocol starting at the silica column binding step. It is also well suited for low-input samples such as buds, where DNA amounts are typically limiting. This approach consistently produces higher yields and cleaner DNA, enabling its use in downstream applications such as genotyping, PCR analysis, Illumina sequencing, or Oxford Nanopore Technologies (ONT) sequencing.

Guidelines

This protocol is intended for **plant tissues that are frozen** and require strong mechanical disruption for efficient DNA extraction.
Follow all **biosafety and chemical safety procedures**, particularly when handling chloroform.
For optimal yield, use **pre-cooled metal adapters** and maintain cryogenic conditions between homogenization steps.
This pre-treatment protocol leads into the **Macherey-Nagel NucleoSpin® Plant II** kit workflow at the silica binding step.

Materials

Materials and Reagents

Liquid nitrogen
Carlson lysis buffer
RNase A
PC buffer
PW1 buffer
PW2 buffer
PE (elution) buffer
Chloroform
1.5 mL microcentrifuge tubes
Green silica spin columns
Collection tubes (2 mL)
Filtered pipette tips
Ethanol (96–100%)
Gloves
Lab coat
Safety goggles

Equipment

TissueLyser II or III
Metal adapter plates for TissueLyser
Vortex mixer
Refrigerated centrifuge (11,000 × g)
Heating block or water bath (55–65 °C)
Chemical fume hood
Nanodrop spectrophotometer
Qubit fluorometer (optional)
Ice bucket
Cryogloves

Safety warnings

- **Liquid nitrogen** is extremely cold and can cause **severe cryogenic burns**. Always handle with **cryogloves and face protection**, and work in a well-ventilated area to avoid oxygen displacement.
- **Chloroform** is toxic, volatile, and potentially carcinogenic. Use only under a **chemical fume hood**, and wear appropriate PPE (gloves, lab coat, goggles).
- **PC Buffer** contains **guanidinium chloride**, a **chaotropic and hazardous agent**. Avoid skin contact and inhalation;
- Ensure that **all ethanol is fully removed** before elution. Residual ethanol can interfere with DNA quantification and downstream enzymatic reactions (e.g., PCR, digestion).
- Avoid excessive vortexing or forceful pipetting during lysis or elution to **preserve high molecular weight DNA**.

Ethics statement

This protocol involves the extraction of DNA from **plant tissues only**. No human or animal subjects are involved. Plant material was collected and processed in accordance with institutional and national guidelines.

Before start

- Pre-cool the **metal adapters** and **tissue samples** in **liquid nitrogen**.
- Preheat a water bath or heat block to **65 °C** for lysis incubation.
- Preheat the **elution buffer (PE)** to **55 °C** for the final elution step.

Prepare the following:
- Carlson lysis buffer
- Wash buffers PW2

Sampling

1 We collected apple buds and apple leaves in the field in 2mL tubes with stainless steel balls. The samples were then flash-frozen and stored at -80°C for long-term conservation.

Carlson Lysis Buffer - Day 0

2 Prepare 400 µL Carlson Lysis buffer per sample:
[M] 100 millimolar (mM) Tris-HCl pH 9.5 ,
[M] 2 Mass / % volume CTAB,
[M] 1.4 Molarity (M) NaCl
[M] 1 Mass / % volume PEG 8000,
[M] 20 millimolar (mM) EDTA.

> Note
>
> The buffer can be prepared in advance by batches of 1 or 2L.
> Prepare the buffer at least one day in advance.

Lysis steps - Day 1 16m

3 Place the frozen samples into pre-cooled metal adapters compatible with the Qiagen TissueLyser II or III.

4 First homogenization round: Run the TissueLyser for 00:00:30 at 22 Hz. 30s

5 Immediately after the first run, immerse both the tube and the metal adapter in liquid nitrogen to re-freeze the sample and maintain cryogenic conditions.

6 ⚠️ Note: If you are processing many samples or working slowly, it may be necessary to refresh the metal adapters in liquid nitrogen between runs to ensure optimal freezing.

7 Second homogenization round: Run the TissueLyser again for 00:00:30 at 22 Hz. 30s

8 After the second homogenization, immediately add 400 µL **of Carlson lysis buffer** to each sample tube.

9 Add 10 µL **of RNase A** (provided with the **Macherey-Nagel NucleoSpin Plant II DNA extraction kit**) to each tube.

10 Mix gently by pipetting up and down or briefly vortexing.

11 Incubate the samples at 65 °C **for** 00:15:00 to facilitate cell lysis and improve DNA yield. 15m

Purification steps 18m 15s

12 Working under a chemical fume hood, add 400 µL of chloroform to each lysed sample tube.

13 Vortex the samples vigorously for 00:00:15 to ensure complete mixing of the aqueous and organic phases. 15s

14 Centrifuge the tubes at 11000 x g, 4°C, 00:10:00 to separate the phases. 10m

15 While centrifuging, prepare the silica spin columns (green) by placing each one into a collection tube.

16 In a separate empty collection tube (without a column), add 450 µL of PC buffer (provided in the Macherey-Nagel kit).

17 After centrifugation, carefully collect the upper aqueous phase (avoid disturbing the interphase and organic layer).

18 Transfer the supernatant into the tube containing 450 µL of PC buffer.

19 Mix thoroughly by pipetting up and down several times.

20 Load 700 µL of the mixture onto the green silica spin column placed in its collection tube.

21 Centrifuge the silica column at 11000 x g, Room temperature, 00:01:00 . 1m

22 Discard the flow-through from the collection tube and place the column back into the same tube.

23 Add 400 µL of PW1 buffer to the column.

24 Centrifuge at 11000 x g, Room temperature, 00:01:00 . 1m

25 Discard the flow-through and return the column to the collection tube.

26 Add 650 µL of PW2 buffer to the column.

27 Centrifuge at 11000 x g, Room temperature, 00:01:00 . 1m

28 Discard the flow-through and return the column to the collection tube.

29 Add 200 µL of PW2 buffer again to the column.

30 Centrifuge at 11000 x g, Room temperature, 00:05:00 to ensure complete removal of residual ethanol. 5m

31 Discard the collection tube and transfer the column to a new, clean 1.5 mL microcentrifuge tube.

DNA Elution

32 Add 50 µL **of pre-warmed elution buffer (PE buffer)** directly to the center of the column membrane.

- Buffer should be **preheated to** 55 °C .

33 Incubate at room temperature for 5 minutes to allow elution.

34 Centrifuge at 11000 x g, Room temperature, 00:01:00 to collect the eluted DNA.

35 (Optional second elution): Pipette the eluate back onto the column membrane.

36 Wait an additional 5 minutes, then centrifuge again at 11000 x g, Room temperature, 00:01:00 .

37 Discard the column, and keep the eluate in the microcentrifuge tube.

DNA Quality and Quantity Assessment

38 Use a **Nanodrop spectrophotometer and/or Qubit fluorometer** to measure DNA concentration and assess purity.

Results

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	Sample	Qubit (ng/µL)	Elution Volume (µL)	Quantity (µg)	Run_name	Flowcell_ID	P2_position	Barcode
	FR_E28_a	160	50	8	251015_Applebuds_F_FRE28-E18-A4-ESC24	PBG23388	B	13
	FR_E18_b	132	50	6.6	251015_Applebuds_F_FRE28-E18-A4-ESC24	PBG23388	B	14
	FR_A4_a	83.4	50	4.17	251015_Applebuds_F_FRE28-E18-A4-ESC24	PBG23388	B	15

	Sample	Qubit (ng/µL)	Elution Volume (µL)	Quantity (µg)	Run_name	Flowcell_ID	P2_position	Barcode
	ES_C24_b	77.2	50	3.86	251015_Applebuds_F_FRE28-E18-A4-ESC24	PBG23388	B	16
	ES_E38_b	67.8	50	3.39	251015_Applebuds_E_ESA23-C28-FRC12-ESE28	PBG23439	A	11
	ES_C28_b	122	50	6.1	251015_Applebuds_E_ESA23-C28-FRC12-ESE28	PBG23439	A	10
	FR_C12_a	139	50	6.95	251015_Applebuds_E_ESA23-C28-FRC12-ESE28	PBG23439	A	12
	ES_A23_b	114	50	5.7	251015_Applebuds_E_ESA23-C28-FRC12-ESE28	PBG23439	A	9

Table 1. DNA quantification and sample information for ONT sequencing.
Qubit-based quantification results for genomic DNA extracted from plant tissue samples using the enhanced pre-treatment protocol prior to the NucleoSpin Plant II workflow. Each sample is listed with its DNA concentration (ng/µL), elution volume (µL), total DNA yield (µg), and associated metadata including run name, flowcell ID, loading position (A/B), and barcode used for multiplexing.
Samples were **multiplexed in groups of four** using a **ligation-based approach** with the **Oxford Nanopore SQK-NBD114.24 kit**, and sequenced on a **FLO-PRO114M flow cell** using the **P2-Solo** device.

40

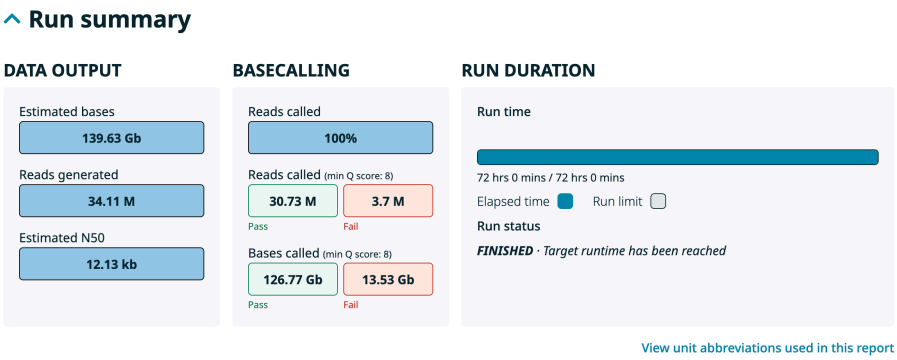

Figure 1. Summary of ONT sequencing run (position A, ES_A23_b; ES_C28_b; ES_E38_b; FR_C12_a samples)
Run performed on a FLO-PRO114M flow cell using the PromethION 2 Solo (P2-Solo) with 4 multiplexed samples (ES_A23_b; ES_C28_b; ES_E38_b; FR_C12_a). A total of **139.63 Gb** of sequence data was generated over **72 hours**, with **34.11 million reads**. Basecalling success reached **100%**, with **126.77 Gb** of high-quality reads (Q ≥ 8). The read N50 was **12.13 kb.**

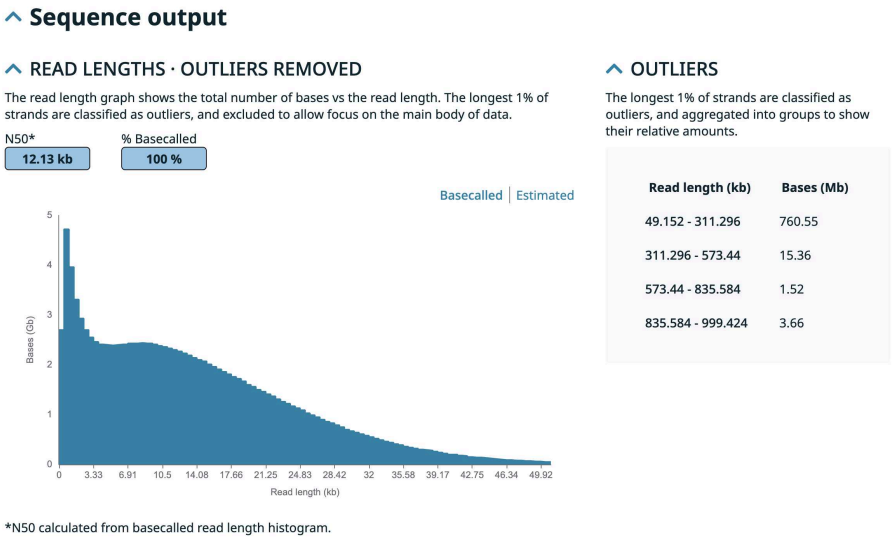

Figure 2. Read length distribution and outlier analysis (position A, ES_A23_b; ES_C28_b; ES_E38_b; FR_C12_a samples)

The read length histogram for run shows a peak between 3–10 kb, with a long tail extending beyond 40 kb. The **N50 of 12.13 kb** confirms a robust proportion of long reads. Outlier analysis reveals several ultra-long reads exceeding **49 kb.**

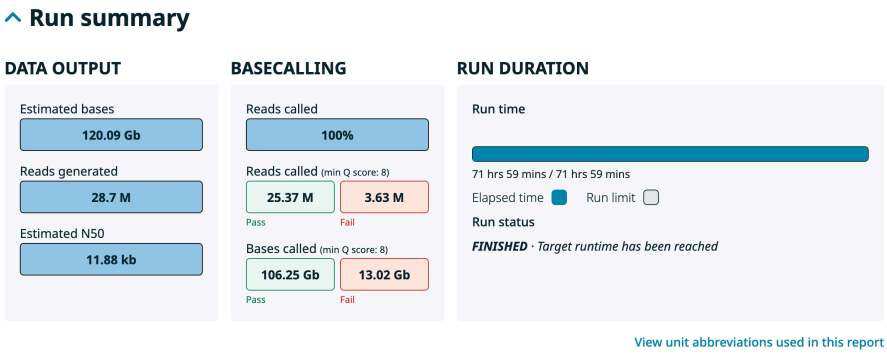

Figure 3. Summary of ONT sequencing run (position B, FR_E28_a; FR_E18_b; FR_A4_a; ES_C24_b samples)

Run performed on the same PromethION 2 Solo device using a second flow cell (FLO-PRO114M), loaded with 4 multiplexed (FR_E28_a; FR_E18_b; FR_A4_a; ES_C24_b). The run yielded **120.09 Gb** of sequence data across **71 hours 59 minutes**, totaling **28.7 million reads**. Of those, **25.37 million reads** (106.25 Gb) passed the Q8 quality threshold. The **N50 was 11.88 kb**, comparable to run A and suitable for downstream long-read analysis.

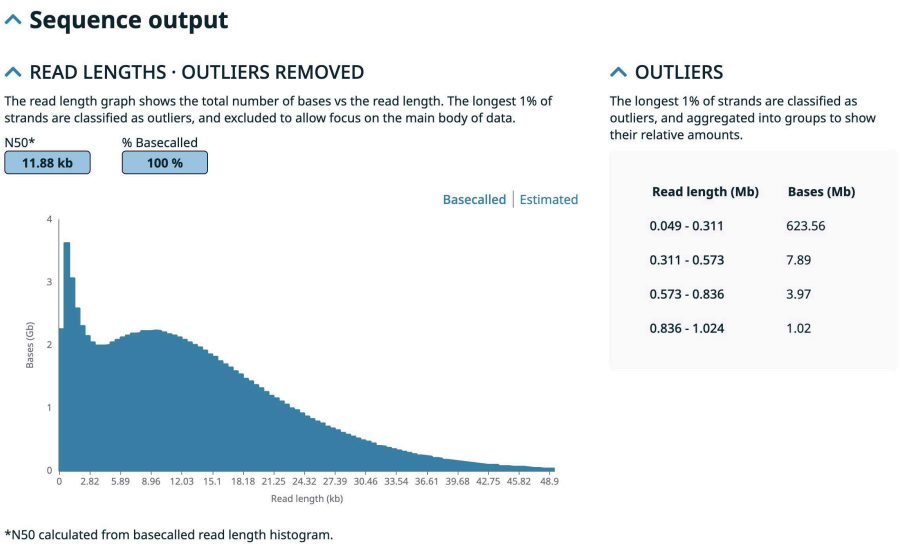

Figure 4. Read length distribution and outlier analysis (position B, FR_E28_a; FR_E18_b; FR_A4_a; ES_C24_b samples)

Read lengths for run P2S-02916-B showed a distribution similar to run A, with a peak between 3–10 kb and an N50 of 11.88 kb.